multiply this difference by 0.365 and divide by the weight of the sample in grams.

(2) *Reagents.* The reagents used are those described in the U.S.P.

(g) *Sulfate in protamine*—(1) *Conduct of the test.* Weigh accurately about 250 milligrams of protamine and dissolve it in about 100 milliliters of approximately tenth-normal hydrochloric acid. Heat to boiling and add 5 milliliters of barium chloride test solution. Digest on a steam bath for 1 hour; allow to cool. Filter through an ignited and weighed Gooch crucible; wash free of chlorides. Dry, ignite, and weight. The weight of barium sulfate thus obtained multiplied by 41.15 and divided by the weight of sample is the percent sulfate (SO_4) in the sample. Calculate the results to a moisture-free basis.

(2) *Reagents.* The reagents used are those described in the U.S.P.

(h) *Nitrogen.* Determine total nitrogen by the method described in the U.S.P., for insulin U.S.P.

(i) *Zinc in insulin-containing solutions or suspensions.* Use the method described in the U.S.P. for insulin injection.

(j) *Zinc in insulin-containing solids.* Dissolve 10 to 20 milligrams, accurately weighed, of insulin-containing solids in 5 to 10 milliliters of distilled water containing one drop of 5*N* hydrochloric acid, and proceed as directed in the U.S.P. under the test for zinc in insulin injection.

[39 FR 11750, Mar. 29, 1974, as amended at 39 FR 40286, Nov. 15, 1974]

Subpart E—Certification

§ 429.40 Requests for certification; samples; storage; approvals preliminary to certification.

(a) A request for certification of a batch is to be addressed to the Food and Drug Administration, Division of Research and Testing (HFD–470), 200 C St. SW., Washington, DC 20204.

(b) The initial request for certification submitted by any person shall be preceded or accompanied by a full statement of the facilities and controls used to maintain the identity, strength, quality, and purity of each batch, including a description of:

(1) The equipment, methods, and processes used in diluting master lots and parts thereof, and in maintaining the identity, strength, quality, and purity of master lots and dilutions therefrom;

(2) The tests and assays made on master lots and mixtures thereof, on dilutions and batches therefrom, and on ingredients used in such dilutions and batches; and

(3) The laboratory facilities used in such controls.

Such initial request shall also be preceded or accompanied by the keys to the master lot marks and batch marks used by such person. When any change is made in any of such facilities or controls, or in any such key, the next request for certification thereafter shall be accompanied by a full statement of such change.

(c) A person who requests certification of a batch shall submit in connection with his request statements showing:

(1) The master lot mark of each master lot used or to be used wholly or partly as an ingredient or component of an ingredient of the batch;

(2) The quantity of each such master lot so used;

(3) The original quantity of each such master lot (unless such information has been previously submitted);

(4) The quantity of the batch; and

(5) The batch mark.

(d) Except as otherwise provided in paragraphs (g) and (h) of this section, a person who requests certification of a batch shall submit in connection with his request and in the quantities hereinafter indicated, accurately representative samples of the following:

(1) The single master lot or the mixture of two or more master lots or parts thereof, to be used as ingredients of the batch; in a quantity containing approximately 10,000 U.S.P. Units of insulin, except that, if the batch is to be isophane insulin suspension, the quantity shall contain not less than 20,000 U.S.P. Units of insulin.

(2) If the batch is to be insulin injection, a trial dilution made from such master lot or mixture, glycerin, phenol or cresol, and hydrochloric acid, which dilution conforms to the standard of identity, strength, quality, and purity

for insulin injection, except that it may contain approximately 40, 80, or 100 units of insulin per milliliter in a quantity containing approximately 5,000 U.S.P. units of insulin.

(3) If the batch is to be protamine zinc insulin suspension, a trial mixture which is intended to be accurately representative of the mixture which will constitute the finished batch; in a quantity containing approximately 10,000 U.S.P. units of insulin.

(4) If the batch is to be protamine zinc insulin suspension or isophane insulin suspension, the lot of protamine used as an ingredient of the trial mixture referred to in paragraph (d)(3) or (7) of this section; in a quantity of approximately 2 grams.

(5) If the batch is to be globin zinc insulin injection, a trial mixture made from the master lot or mixture referred to in paragraph (d)(1) of this section, globin, zinc chloride, hydrochloric acid, glycerin, and phenol or cresol, which mixture is intended to be accurately representative of the mixture which will constitute the finished batch; in a quantity containing approximately 10,000 U.S.P. units of insulin.

(6) If the batch is to be globin zinc insulin injection, the lot of globin hydrochloride from which the globin is to be prepared for use as an ingredient of the trial mixture referred to in paragraph (d)(5) of this section; in a quantity of approximately 5 grams.

(7) If the batch is to be isophane insulin suspension, a trial mixture which is intended to be accurately representative of the finished batch; in a quantity of approximately 10,000 U.S.P. units of insulin.

(8) If the batch is to be insulin zinc suspension, prompt insulin zinc suspension, or extended insulin zinc suspension, a trial mixture which is intended to be accurately representative of the finished batch; in a quantity of approximately 50 milliliters.

(9) The finished batch; for all tests except sterility, not less than 10 retail packages.

(10) The finished batch for sterility testing, 20 retail packages, collected at approximately equal intervals throughout each filling operation (as defined by the U.S.P.), except that if it is insulin injection containing 500 U.S.P. Units of insulin per milliliter, in lieu of the volume contained in the retail package each such container may contain an amount of drug that is less than that contained in the retail package but in no case less than 5 milliliters.

(e) Except as otherwise provided by paragraphs (g) and (h) of this section, a person who requests certification shall submit in connection with his request results of the tests and assays listed after each of the following materials, made by him on a sample of such material:

(1) The master lot or mixture, referred to in paragraph (d)(1) of this section: Ash, nitrogen, potency, pH, sterility, and zinc, if such master lot or mixture is a solution; ash, moisture, nitrogen, potency, and zinc, if such master lot or mixture is a solid.

(2) A trial dilution of such master lot or mixture, of the potency of the trial dilution referred to in paragraph (d)(2) of this section: Nitrogen, pH, and potency.

(3) If the batch is to be protamine zinc insulin suspension, the trial mixture referred to in paragraph (d)(3) of this section: Nitrogen, pH, zinc, and biological reaction (by the test prescribed in the U.S.P.).

(4) If the batch is to be protamine zinc insulin suspension or isophane insulin suspension, the protamine referred to in paragraph (d)(4) of this section: Moisture, nitrogen, and sulfate.

(5) If the batch is to be globin zinc insulin injection the trial mixture referred to in paragraph (d)(5) of this section: Nitrogen, pH, zinc, and biological reaction (by the test prescribed in the U.S.P.).

(6) If the batch is to be globin zinc insulin injection, the globin hydrochloride referred to in paragraph (d)(6) of this section: Moisture, nitrogen, chloride, and ash.

(7) If the batch is to be isophane insulin suspension, the trial mixture referred to in paragraph (d)(7) of this section: Nitrogen, pH, zinc, isophane ratio of the protamine to the master lot or mixture (by the test prescribed in § 429.30(c)), and biological activity of the supernatant liquid (by the test prescribed in the U.S.P.).

(8) If the batch is to be insulin zinc suspension, prompt insulin zinc suspension, or extended insulin zinc suspension, the trial mixture referred to in paragraph (d)(8) of this section: Nitrogen, pH, zinc, zinc in the supernatant liquid and insulin not extracted by buffered acetone solution.

(9) The finished batch: Nitrogen, pH, sterility; and if the batch is protamine zinc insulin suspension, globin zinc insulin injection, isophane insulin suspension, insulin zinc suspension, prompt insulin zinc suspension, or extended insulin zinc suspension, zinc.

(f) The results of tests and assays for the following shall be reported in the terms indicated:

(1) Ash (except globin hydrochloride)—milligrams per 1,000 U.S.P. Units of insulin.

(2) Ash in globin hydrochloride—percent by weight.

(3) Chloride—percent by weight as HCl.

(4) Insulin not extracted by buffered acetone solution—percent of total nitrogen of the preparation not extracted by buffered acetone solution.

(5) Isophane ratio—milligrams of protamine per 100 U.S.P. Units of insulin.

(6) Moisture—percent by weight.

(7) Nitrogen (except in globin hydrochloride and protamine)—milligrams per milliliter in the cases of solutions and suspensions, and percent by weight in the case of solids.

(8) Nitrogen in globin hydrochloride—percent by weight, calculated to a moisture-free, ash-free, chloride-free basis.

(9) Nitrogen in protamine—percent by weight, calculated to a moisture-free basis.

(10) Potency—U.S.P. Units of insulin per milliliter in the case of solutions, and U.S.P. Units of insulin per milligram in the case of solids.

(11) pH.

(12) Sulfate—percent by weight as SO_4, calculated to a moisture-free basis.

(13) Zinc—milligrams per milliliter in the cases of solutions and suspensions, and percent by weight in the case of solids.

(g)(1) No sample referred to in paragraphs (d) (1) to (3), inclusive, of this section, and no result referred to in paragraphs (c) (1) to (8), inclusive of this section, is required if such sample or result has been submitted in connection with a previous request for certification. Except for paragraphs (d) (9), (10), and (e)(9), the samples referred to in paragraph (d) of this section and the results referred to in paragraph (e) of this section for insulin injection, protamine zinc insulin suspension, globin zinc insulin injection, or isophane insulin suspension are not required if the Commissioner has previously approved a trial mixture containing 40, 100 units of insulin per milliliter or trial dilution containing approximately 40, 100 units of insulin per milliliter and the mixture or dilution was prepared from the same materials and in the same manner, except for adjustment of pH of the buffer solution.

(2) Each sample submitted pursuant to this section shall be so packaged as to maintain its representative character, and in the case of any solution or suspension, shall be collected and packaged under aseptic conditions. Each package shall be clearly identified as to its contents and shall bear the name and post office address of the person submitting the request.

(3) The packages constituting the samples submitted pursuant to paragraph (d)(9) of this section shall be collected at such intervals that the quantities packaged between collections are approximately equal; in no case shall any such quantity be more than 10,000 packages. The collections shall cover the entire period of packaging.

(4) Each sample submitted pursuant to paragraphs (d) (2), (3), (5), (7), and (8) of this section shall be accompanied by a statement showing the identity, quality, and quantity of each substance used as an ingredient or as a component of an ingredient in the material from which the sample was taken.

(5) If the tests and assays, results of which are submitted pursuant to paragraph (e)(2) of this section, were not made on the same trial dilution as that from which the sample submitted pursuant to paragraph (d)(2) of this section was taken, such sample shall be accompanied by a statement showing the identity, quality, and quantity of each substance used as an ingredient or as a

component of an ingredient of the trial dilution on which such tests and assays were made.

(6) The value for nitrogen submitted pursuant to paragraphs (e) (1) and (2) of this section may be calculated from the result of a test therefor submitted pursuant to either paragraph (e) (1) or (2) of this section. The result on potency required under paragraph (e)(1) of this section may be calculated from an assay therefor submitted pursuant to paragraph (e)(2) of this section. The value of each of the components nitrogen and zinc, to the extent required under paragraph (e)(9) of this section, may be calculated from the result of a test therefor submitted pursuant to paragraph (e) (3), or (5), or (7) or (8) of this section or from the result of a test of the bulk dilution from which the batch was prepared. The value for nitrogen required under paragraph (e)(9) of this section may, if the batch is insulin injection, insulin zinc suspension, prompt insulin zinc suspension, or extended insulin zinc suspension, be calculated from a test therefor submitted pursuant to either paragraph (e) (1) or (2) of this section. Each calculated value shall be indicated as such.

(7) The information required under paragraphs (c) (1), (2), and (3) of this section, and the samples and results of tests and assays required under paragraphs (d) (1) and (2) and (e) (1) and (2) of this section, should be submitted before submission of the samples and results required in paragraphs (d) (3) to (8), inclusive, of this section and (e) (3) to (8), inclusive, of this section; and the samples and results required under paragraphs (d) (3) to (8), inclusive, and (e) (3) to (8), inclusive, should be submitted before submission of the information, samples, and results required under paragraphs (c) (4) and (5), (d) (9) and (10), and (e)(9) of this section. All information, including results of tests and assays (except results of tests for sterility), required under this section should be submitted at the same time as the samples to which they relate are submitted.

(h) The person who requests certifications shall submit such information additional to that submitted pursuant to paragraphs (b), (c), (e), and (g) of this section, such additional samples of any substance referred to in paragraph (d) of this section, and such samples of any other substance used or to be used as an ingredient or as a component of an ingredient in the batch, as the Commissioner may require for the purpose of investigations to determine whether or not such batch complies with the requirements set forth by § 429.41 for the issuance of a certificate.

(i) After a sample required by paragraph (d) of this section is taken from any master lot or mixture of part of two or more master lots, such master lot or master lots and all parts thereof, and all dilutions and batches and all parts thereof in which any such master lot is used as an ingredient or as a component of an ingredient, shall be stored at the establishment where manufactured until used up or shipped or otherwise delivered, at a temperature above freezing but not above 15° C. (59° F.), and under such other conditions as prevent, so far as practicable, any change in composition; except that master lots and parts thereof which are solids may be stored at ordinary room temperatures.

(j) As promptly as practicable after the samples submitted pursuant to paragraphs (d) (1) and (2) of this section, and any other material or information relative thereto that may be required under this section, are received by the Commissioner, he shall notify the person who submitted such samples of his approval or refusal to approve the use of the master lot or mixture for the making of bulk dilutions. In case of a refusal to approve, the Commissioner shall state his reasons therefor.

(k) In like manner, the Commissioner shall notify the person who submits samples pursuant to paragraphs (d) (3) to (8), inclusive, of this section of his approval or refusal to approve the use of the materials represented by such samples in completing the manufacture of the batch. In case of a refusal to approve, the Commissioner shall state his reasons therefor.

(l) If, under the provisions of paragraph (j) or (k) of this section, the Commissioner has refused to approve any material for use in a subsequent operation, he shall examine no other

sample required hereunder which includes such material as an ingredient or component of an ingredient, unless and until the person requesting certification makes an adequate showing that the cause for such refusal no longer exists.

[39 FR 11750, Mar. 29, 1974, as amended at 39 FR 40286, Nov. 15, 1974; 44 FR 48968, Aug. 21, 1979; 44 FR 55170, Sept. 25, 1979; 45 FR 40111, June 13, 1980; 50 FR 8996, Mar. 6, 1985; 55 FR 11582, Mar. 29, 1990]

§ 429.41 Certifications.

(a) If it appears to the Commissioner, after such investigation as he considers necessary, that:

(1) The information (including results of tests and assays) and the samples required by or pursuant to § 429.40 have been submitted, and such information contains no untrue statement of a material fact;

(2) The batch complies with the regulations in this part 429 and conforms to the standards of identity, quality, strength, and purity for insulin injection, protamine zinc insulin suspension, globin zinc insulin injection, isophane insulin suspension, insulin zinc suspension, prompt insulin zinc suspension, or extended insulin zinc suspension;

the Commissioner shall certify that such batch is safe and efficacious for use, subject to such conditions on the effectiveness of such certifications as are set forth in § 429.45, and shall issue to the person who requested it a certificate to that effect.

(b) If the Commissioner determines, after such investigation as he considers to be necessary, that the information submitted pursuant to § 429.40 or the batch covered by such request, does not comply with the requirements set forth in paragraph (a) of this section for the issuance of a certificate, the Commissioner shall refuse to certify such batch and shall give notice thereof to the person who requested certification, stating his reasons for refusal.

(c) Upon the request of the manufacturer, the Commissioner shall certify as a ''batch'' a master lot, which has been approved in accordance with § 429.40(j) as safe and efficacious for use in preparation of an insulin-containing drug, subject to the conditions on the effectiveness of such certifications as are set forth in § 429.45(a) (1) and (b) (4).

(d) For the purposes of his investigations under the authority of this section, the Commissioner may accept, when he is satisfied as to the completeness and accuracy thereof, the results of any tests or assays made by the control laboratory of the Insulin Committee of the University of Toronto.

§ 429.45 Conditions on the effectiveness of certificates.

(a) A certificate shall not become effective:

(1) If it is obtained through fraud, or through misrepresentation or concealment of a material fact.

(2) With respect to any package, unless its immediate container complies with the requirements of § 429.10 and such package or such immediate container has been so sealed that its contents cannot be used without destroying such package or seal.

(3) With respect to any package, unless its label and labeling bear all words, statements, and other information, and are distinguished by the color or colors, required by §§ 429.11 and 429.12.

(b) A certificate shall cease to be effective: (1) With respect to any package of insulin injection, protamine zinc insulin suspension, globin zinc insulin injection, isophane insulin suspension, insulin zinc suspension, prompt insulin zinc suspension, or extended insulin zinc suspension on the expiration date specified in the U.S.P.

(2) With respect to any package, when such package or the seal thereof or the immediate container therein or the seal of the immediate container is broken, or when its label or labeling ceases to conform to any requirement of § 429.11 or § 429.12.

(3) With respect to any package, when the drug therein so changes that it fails to meet the standards of identity, strength, quality, and purity upon the basis of which the batch was certified; except that those minor changes in potency (not exceeding 10 percent from the potency stated on the label, in the case of insulin injection) which occur before the expiration date, and which are normal and unavoidable in